Pergamon

Tetrahedron Letters, Vol. 37, No. 7, pp. 1065-1068, 1996

0040-4039/96 $15.00 + 0.00

0040-4039(95)02349-6

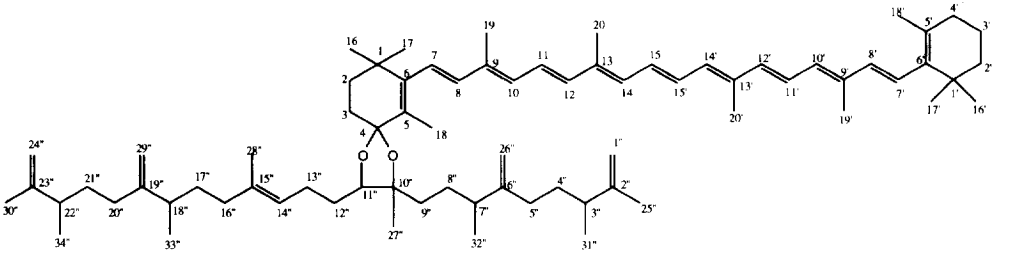
Botryoxanthin A, A Member of a New Class of Carotenoids from the Green Microalga *Botryococcus braunii* Berkeley

Shigeru Okada,* Hisashi Matsuda, Masahiro Murakami and Katsumi Yamaguchi

Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo, 113, Japan

Abstract: Botryoxanthin A, a member of novel natural carotenoids consisting of ketocarotenoid and squalene analog, was isolated from the Berkeley strain of the green microalga *Botryococcus braunii*. Its structure was determined to be **1** on the basis of 2D NMR data.

Botryococcus braunii is a green colonial microalga which produces unusually high levels of hydrocarbons[1]. Strains of this alga are divisible into at least three races, A, B, and L, by distinct types of hydrocarbons[2,3,4]. The color of some strains belonging to the B and L races turns red when they are in the stationary phase or under nitrogen-deficient conditions, because ketocarotenoids such as canthaxanthin and/or echinenone accumulate in the extracellular matrix[5,6]. The Berkeley (Showa) strain[7] is one of the B race which produces branched triterpenoids called botryococcenes[8] and squalene-related compounds such as tetramethylsqualene[9]. The extracellular matrix of this strain also changes from green to orange under certain conditions. Even in the green colonies, however, we have detected unknown carotenoids which we named botryoxanthins. In this paper, we report the isolation and structural elucidation of one of them (**1**).



1

B. braunii Berkeley was cultured with modified Chu13 medium[2,10]. Cultures were grown under illumination of 240μE/m^2·s on a 12L:12D cycle at 25°C, aerated with sterile air containing 2% CO_2.

The freeze-dried algal cells (77.1g) were sonicated in acetone. The acetone extract was subjected to silica gel column chromatography and a fraction eluted with *n*-hexane/benzene (2:1) was collected. This fraction was

subjected to ODS column chromatography and a yellow band was eluted with MeCN/EtOAc (1:1). The yellow material was subjected to normal-phase HPLC (Nomura Chemical Develosil Silica 60-5) with *n*-hexane/diethyl ether (100:1) followed by reversed-phase HPLC on Nakarai Tesk Cosmosil ODS-AR with MeCN/acetone (4:6) using a recycle valve, and pure botryoxanthin A (**1**, 13mg) was obtained after five recycles.

The molecular formula of **1** was determined to be $C_{74}H_{112}O_2$ by HRFABMS [m/z 1032.8690 $(M)^+$ Δ 2.7 mmu] and NMR data (Table 1). This compound has a β,β–carotene type UV-VIS absorption in hexane [λ_{max} 479 (ε=0.92x10^5), 450 (ε=1.04x10^5), 426(sh), 274 (ε=0.19x10^5)]. The ^{1}H and ^{13}C NMR spectra

Table 1. ^{1}H and ^{13}C NMR Spectra of Botryoxanthin A in C_6D_6

Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)	Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)
1		34.97(s)	3, 16, 17	1"	4.81(brs) 4.84(brs)	110.23(t)	3", 25"
2	1.77(m) 2.01(m)	36.90(t)	3, 16, 17	2"		149.74(s)	3", 4", 31"
3	2.07(m) 2.23(m)	34.68(t)		3"	2.19(m)	41.56(d)	1", 4", 25", 31"
4		107.40(s)	2, 3, 18	4"	1.50(m) 1.63(m)	33.75(t)	3", 5", 31"
5		131.94(s)	7, 18	5"	2.05(m)	32.56(t)	3", 4", 7", 26"
6		143.76(s)	8, 16, 17, 18	6"		154.74(s)	5", 7", 8", 32"
7	6.21(d, 15.9)	126.33(d)	8	7"	2.13(m)	40.98(d)	8", 26", 32"
8	6.35(d, 15.9)	139.81(d)	10, 19	8"	1.50(m) 2.01(m)	29.78(t)	7", 9", 32"
9		135.48(s)	7, 8, 11, 19	9"	1.20(m) 1.82(m)	33.34(t)	7", 11", 27"
10	6.26(d, 11.5)	132.68(d)	8, 12, 19	10"		81.75(s)	11", 27"
11	6.74(dd, 11.5, 15.0)	125.33(d)	10	11"	4.06(dd, 2.7, 10.0)	86.84(d)	13", 27"
12	6.47(d, 15.0)	138.42(d)	10, 14, 20	12"	1.41(m) 1.79(m)	30.60(t)	13", 14"
13		136.57(s)	11, 15, 20	13"	2.27(m) 2.45(m)	26.16(t)	11", 14"
14	6.32(m)	133.46(d)	12, 15, 20, 15'	14"	5.30(m)	124.33(d)	13", 28"
15	6.68(m)	130.77(d)	14	15"		136.13(s)	13", 16", 17", 28"
16	1.10(s)	29.19(q)		16"	2.05(m)	37.99(t)	14", 17", 18", 28"
17	1.09(s)	27.40(q)		17"	1.44(m) 1.68(m)	34.40(t)	16", 18", 33"
18	2.15(s)	15.30(q)	3	18"	2.12(m)	40.06(d)	16", 17", 29", 33"
19	1.88(s)	12.72(q)	8, 10	19"		154.66(s)	17", 18", 20", 33"
20	1.85(s)	12.86(q)	12, 14	20"	1.98(m)	32.01(t)	18", 21", 22", 29"
1'		34.57(s)	2', 3', 16', 17'	21"	1.45(m) 1.58(m)	33.77(t)	20", 22", 34"
2'	1.49(m)	39.96(t)	4', 16', 17'	22"	2.14(m)	41.38(d)	20", 21", 24", 30"
3'	1.59(m)	19.70(t)	2', 4'	23"		149.71(s)	21", 22", 30", 34"
4'	1.98(m)	33.34(t)	2', 18'	24"	4.79(brs) 4.80(brs)	110.16(t)	22", 30"
5'		129.38(s)	3', 4', 7', 18'	25"	1.63(d)	18.88(q)	1", 3"
6'		138.37(s)	2', 4', 8',16', 17', 18'	26"	4.92(brs) 4.94(brs)	108.08(t)	5", 7"
7'	6.32(m)	126.87(d)	8'	27"	1.24(s)	22.81(q)	11"
8'	6.38(d, 15.9)	138.65(d)	10',19'	28"	1.64(d)	16.22(q)	14", 16"
9'		135.99(s)	7', 8', 11', 19'	29"	4.87(brs) 4.89(brs)	108.01(t)	18", 20"
10'	6.33(d, 11.4)	131.89(d)	8', 12', 19'	30"	1.59(d)	18.89(q)	22", 24"
11'	6.77(dd, 11.4, 15.0)	125.52(d)	10'	31"	1.03(d, 6.7)	20.04(q)	3", 4"
12'	6.47(d, 15.0)	137.98(d)	10', 14', 20'	32"	1.12(d, 6.9)	20.60(q)	7", 8"
13'		136.77(s)	11', 15', 20'	33"	1.05(d, 7.0)	20.42(q)	17", 18"
14'	6.30(m)	133.19(d)	15, 12', 15', 20'	34"	1.00(d, 7.0)	19.97(q)	21", 22"
15'	6.67(m)	130.58(d)	14'				
16'	1.15(s)	29.19(q)	2'				
17'	1.15(s)	29.19(q)	2'				
18'	1.81(s)	21.99(q)	4'				
19'	1.92(s)	12.81(q)	8', 10'				
20'	1.86(s)	12.84(q)	12', 14'				

also indicated presence of β, β–carotene type carotenoid moiety. In one β–end group, the COSY45 clearly gave connectivities of three methylenes from C-2' to C-4' [2'-H_2 (δ_H 1.49), 3'-H_2 (δ_H 1.59) and 4'-H_2 (δ_H 1.98)]. Moreover, the HMBC correlations of 16',17'-Me to C-1', 16',17'-Me to C-2', 4'-H_2 to C-5', 18'-Me to C-5', and 18'-Me to C-6' established the structure of β–end group straightforwardly (Fig. 1). On the other hand, another β–end group showed connectivity between two methylenes [2-H_2, (δ_H 1.77, 2.01) and 3-H_2 (δ_H 2.07, 2.23)] in COSY 45 and the connectivities of remaining part of this β–end group were also deduced by HMBC. Newly generated quaternary carbon C-4 was confirmed to be ketal carbon from the chemical shift (δ_C 107.4). The conjugated polyene part was assigned by COSY45, HMQC, HMBC. The coupling constants between 7-H and 8-H, 7'-H and 8'-H, 11-H and 12-H, and 11'-H and 12'-H were 15.9 or 15.0, though that between 15-H and 15'-H was not decided for complexity of the signals. The NOESY spectrum showed correlations between 7-H and 19-Me, 8-H and 10-H, 11-H and 20-Me, 12-H and 14-H, 14-H and 15'-H, 7'-H and 19'-Me, 8'-H and 10'-H, 11'-H and 20'-Me, and 12'-H and 14'-H. Moreover, this compound did not show any *cis*-peak in UV-VIS spectrum. Therefore geometries of the conjugated polyene part were thought to be all-*trans*.

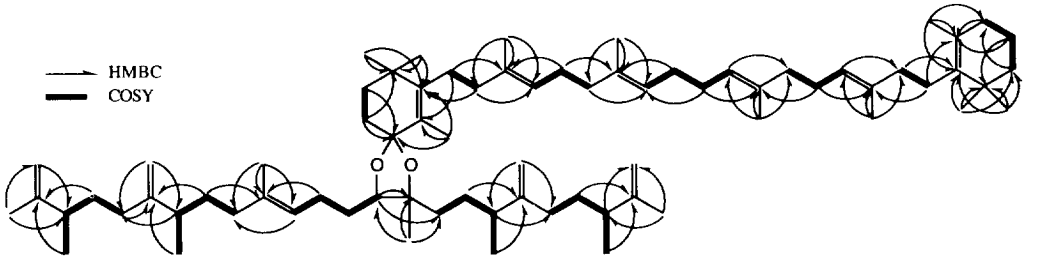


Fig.1 COSY45 and HMBC correlations of botryoxanthin A

Inspection of ^1H and ^{13}C-NMR spectra, COSY45, HOHAHA, HMQC, and HMBC indicated that the remaining part of **1** was very close to tetramethylsqualene obtained from the same strain of *B. braunii*[8]. But a sp^3 quaternary carbon (C-10", δ_C 81.75) and a sp^3 methine [11"-H, (δ_H 4.06), C-11", (δ_C 86.84)], which were thought to attach to oxygen from the chemical shifts, existed instead of a sp^2 quaternary carbon and a sp^2 methine in tetramethylsqualene. The connectivities of the down-fielded methine (11"-H) with 12"-H_2 and C-10" were confirmed by COSY45 and HMBC, respectively. The connectivities of C-10" with 27"-Me and C-9" were also deduced from HMBC. The configuration of the double bond between C-14" and C-15" was thought to be *E* because the NOESY spectrum showed correlations between 14"-H and 16"-H_2, and 13"-H_2 and 28"-Me. Thus, whole structure of botryoxanthin A is β, β–carotene connecting with C-10" and C-11" of tetramethylsqualene at C-4 ketal carbon.

The occurrence of carotenoid glycosides and higher carotenoids consisting of more than eight isoprenoid units has been known. However, botryoxanthin A may be the first example of a new class of carotenoids that are composed of a C_{40}-carotenoid and a squalene-related compound. Subsequent discovery of similar carotenoids can be expected. Botryoxanthin A free from chlorophylls and intracellular carotenoids such as lutein could be extracted without breaking the cell wall. This implies that botryoxanthin A may exist in the extracellular matrix like other secondary carotenoids and contribute to the color expression of the algal colonies.

Furthermore, botryoxanthin A seems to be closely related to the hydrocarbon production because squalene-related compounds have so far been detected only in the strains producing botryococcenes[9,11]. It suggests that botryoxanthin A may be used as a chemotaxonomic index for strains of *B. braunii*.

Acknowledgments. We are grateful to Prof. H. Iwamoto, Meiji University for donating the Berkeley strain. We are also indebted to Drs. T. Okino and Y. Nakao for measuring HRFABMS. This study was partly supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

1. Brown, A. C.; Conway, E. *Phytochemistry* **1969**, 8, 543-547 .
2. Metzger, P.; Berkaloff, C.; Casadevall, E.; Coute, A. *Phytochemistry* **1985**, 24, 2305-2312.
3. Metzger, P.; Templier, J.; Largeau, C.; Casadevall, E. *Phytochemistry* **1986**, 25, 1869-1872.
4. Metzger, P.; Allard, B.; Casadevall, E.; Berkaloff, C.; Coute, A. *J. Phycol.* **1990**, 26, 258-266.
5. Grung, M.; Metzger, P.; Liaaen-Jensen, S. *Biochem. Syst. Ecol.* **1989**, 17, 263-269.
6. Grung, M.; Metzger, P.; Berkaloff, C.; Liaaen-Jensen, S. *Comp. Biochem. Physiol.* **1994**, 107B, 265-272.
7. Nonomura, A. M. *Jap. J. Phycol.* **1988**, 36, 285-291.
8. Wolf, F. R.; Nonomura, A. M.; Bassham, J. A. *J. Phycol.* **1985**, 21, 388-396.
9. Huang, Z.; Poulter, C. D. *Phytochemistry* **1989**, 28, 1467-1470.
10. Largeau, C.; Casadevall, E.; Berkaloff, C.; Dhamelincourt, P. *Phytochemistry* **1980**, 19, 1043-1051.
11. Metzger, P.; Casadevall, E. *Tetrahedron Lett.* **1983**, 24, 4013-4016.

(*Received in Japan* 11 *November* 1995; *accepted* 7 *December* 1995)